EXPERIMENTAL EPIDEMIOLOGY.

I. An Artificially Induced Epidemic of Mouse Typhoid.

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The problem set ourselves was the study of an epidemic of mouse typhoid conducted under experimental conditions. The painstaking observations recorded in Dr. Lynch's paper,[1] far exceeding those usually accorded outbreaks of disease among domesticated animals, seemed to put rather than to answer questions of epidemiology. It appeared to us therefore that an epidemic started under fixed conditions, in which one person followed the happenings day by day and recorded the events, would not only tend to eliminate errors traceable to the elements of surprise and lack of preparation, but also by providing a more homogeneous material yield results of greater consistency, while the two major factors of host and parasite would be placed under highly favorable conditions of control.

Method.

Of laboratory mammals, mice are most easily assembled and observed in large numbers. Moreover, they are subject to a bacterial infection, mouse typhoid, of gastrointestinal origin, the pathology of which is quite well known. The lesions found in and characteristic of the disease affect several important viscera and are obvious to the unaided eye. The bacteriology also is sufficiently worked out so as to serve as a guide in what may be regarded as doubtful instances. Moreover, as is well known, the disease mouse typhoid constitutes a common sporadic fatal epidemic affection among mice and even from time to time sweeps through mice colonies in highly destructive waves.

[1] Lynch, C. J., *J. Exp. Med.*, 1922, xxxvi, 15.

In view of the experiences described in Dr. Lynch's paper,[1] our experimental investigations were started with mouse typhoid. While under way, Topley's[2] series of valuable papers on the same general topic began to appear in print. They will be discussed along with the deductions from our own observations in the proper place.

The procedure adopted by us at the outset was one chosen to simulate the conditions of epidemic outbreaks of disease not only among carefully segregated small domestic animals but also those which occur among human beings.

Thus what may be termed a mouse village was set up by placing in rows on metal shelves metal cages 7 by 10 by 5 inches with wire mesh tops. 5 mice were placed in each cage without communication between the cages, so that infection could be transferred only by the hands and implements of the person cleaning the cages and feeding the mice. Great care was taken throughout the experiment to exclude roaches, ants, flies, and other insects and vermin from the room. The temperature of the room was kept constantly at 68°F. whenever the outside temperature was below this. In the summer months the temperature of the room was, of course, higher. The cages were thoroughly cleaned once each week, always in the same order, and the mice were fed in the same order daily as follows: grain in the morning and bread moistened with milk in the afternoon. Precautions were taken to exclude extraneous disease. The mice used came from a carefully controlled healthy stock bred in the Institute and free of communicable disease.

There are certain advantages in keeping the experimental animals in small groups: it simplifies identification and stock taking, and the keeping of records; and allows frequent inspection for dead animals; it reduces the death rate resulting from fighting. The spread of infection is limited to one agent; *viz.*, the cleaning implements and hands of the caretaker.

Introduction of the Virus.—As already stated, when the study was begun Topley's papers had not appeared. His method of placing a known amount of culture on small bits of bread to be consumed by mice from which food had been withheld for 24 hours is efficacious

[2] Topley, W. W. C., *J. Hyg.*, 1920–21, xix, 350.

and more accurate than our first attempts by allowing hungry mice to drink milk from a tube. In order to give the exact dose to each mouse, we later introduced the bacteria suspended in milk directly into the stomach through a small silver tube. In this way the dose can be accurately measured and the use of more than 1 cage for each 5 mice obviated for the preliminary step of infecting the first mice.

Inspection.—The cages were examined three times daily except Sunday, when only one inspection was made. Even under these conditions it sometimes happens, especially on the first round of the day, that only a partially eaten body of a mouse is found. The survivors quickly attack the body of a dead mouse and devour the softer parts. Earlier in the experiments, when we had little experience, the number of deaths which could not be definitely determined as due to *Bacillus typhi murium* infection was proportionately larger than in the later series.

The bodies collected at 9 a.m. rounds were autopsied at 10 a.m. Those collected later were kept in the refrigerator (+4°C.) until the following morning.

Records.—In following the progress of the infection, two charts were kept: (*a*) plot of the total number of deaths for the whole series, according to days, and (*b*) spot map using colored pins showing the location of the mice which died and the number of days elapsed since the beginning of the exposure.

Autopsy Technique.—The notes at autopsy included the weight of the body and of the spleen, and a brief description of the macroscopic appearances of the spleen, intestines, liver, and gall bladder. The bacteriological examinations of the spleen, intestinal contents, and gall bladder were carried out in the following manner. A small bit of the material was transferred to a tube containing 5 cc. of brilliant green broth (brilliant green 1:200,000 in broth of pH 7.4) and crushed inside the tube. To the tube there was then added 0.25 cc. of a 1 per cent sterile aqueous solution of lead acetate. The tube was incubated at 37°C. over night. If on the following morning the precipitate in the tube was brown, showing evidence of sulfide production (presumptive test of the presence of *Bacillus typhi murium*), a loopful of the broth was smeared on lactose-saccharose-neutral red agar, pH 7.4, plates containing 1:400,000 brilliant green. Colonies from these

plates were fished into lead acetate agar tubes containing four sugars:[3] lactose, raffinose, saccharose, and salicin, 0.25 per cent of each. Cultures having the characteristic reactions of *Bacillus typhi murium* were then agglutinated with two monotypical immune sera. The longer incubation period for brilliant green broth tubes has revealed a higher percentage of deaths attributable to mouse typhoid than was obtained with the usual procedure of 1 hour's incubation in a water bath.[4] The addition of lead acetate to the broth greatly reduces the number of brilliant green plates required. The first few hundred examinations with this technique were controlled by the usual culture method resulting in a gain of about 10 per cent in the positive findings. The lead acetate did not fail in a single instance to reveal *Bacillus typhi murium*, and in 125 tubes, in which there was no browning controlled by plating, no *Bacillus typhi murium* was found. Many brilliant green tubes containing feces may show the sulfide reaction when no *Bacillus typhi murium* is present, due to the growth of other sulfide-producing organisms such as *Bacillus alkaligenes*, etc. The plating on brilliant green and transferring of the colonies to the four sugar tubes control this point.

The final agglutination test is highly important as endemic strains, differing antigenically from the strain used to induce the artificial infection, are sometimes found in mice succumbing in course of the epidemic experimentally set up.

[3] The composition of this medium is as follows:

Beef extract	3 gm.
Peptone	10 "
NaCl	5 "
Agar	15 "
Lactose	0.25 per cent.
Raffinose	0.25 " "
Saccharose	0.25 " "
Salicin	0.25 " "
Andrade indicator	1 " "
Distilled water	1,000 cc.

pH 7.2

Lead acetate ... 1 cc. of 0.25 per cent solution to each 4 cc. tube of medium.

[4] Undoubtedly in our earlier series many negative results were recorded which with the newer technique would have been found positive.

Bacillus typhi murium Employed.

A bacillus conforming to the cultural characteristics of *Bacillus typhi murium* and pathogenic for mice was isolated from an epizootic among a cancer breeding stock.[1] This strain (Mouse Typhoid I) was passed by intraperitoneal injection through 5 mice. A suspension containing 1:20 of an 18 hour culture on slant agar was then fed to a mouse, and on the death of this animal the bacillus recovered, after being identified culturally as belonging to the same group, was fed to 4 mice in succession. As it happened, later immunological tests showed the strain recovered from the final mouse of this series to be antigenically different from the original strain but having identical fermentative properties with those of so called *Bacillus typhi murium* and being pathogenic for mice. It is probable that this strain which we term Mouse Typhoid II was enzootic among the stock used in the preliminary experiments, replacing somewhere in the series of 4 mice the strain used originally to induce infection. It came out in later studies that the second large outbreak among the cancer breeding stock[1] was caused by a strain antigenically identical with this strain. However this may be, the strain acquired highly invasive powers, as the experiments to be described will show. From this point on, this strain appeared in the animals succumbing to the mouse typhoid arising in the course of our experiments except in a few instances in which an immunologically different strain was obtained in culture. The fact of this substitution of strains emphasizes the importance of making regular immunological tests on all strains employed in and recovered during the experiments.

In referring to this bacillus with which our experiments were conducted as *Bacillus typhi murium*, we would have it understood that it belongs to the class of bacilli embraced under this term and was later found to be indistinguishable by immunological tests from *Bacillus pestis caviæ* of the paratyphoid B group. The immunological reactions of the bacillus will be described in a separate communication.[5,6]

Preliminary Culture Feeding.

Feeding Series B.—November 2, 1919. 18 normal mice, after fasting 24 hours, were fed during 24 hours with milk containing 1:20 culture of Mouse Typhoid II

[5] Amoss, H. L., and Haselbauer, P. P., *J. Exp. Med.*, 1922, xxxvi, 107.

[6] Webster, L. T., *J. Exp. Med.*, 1922, xxxvi, 97.

from an 18 hour growth on slant agar. During the next 18 days, 15 died and yielded positive cultures of the same bacillus in the feces and spleen; in 8 the bacillus was recovered also from the gall bladder. The deaths took place as follows: 3 occurred in 7 days; 4 in 8 days; 2 in 9 days; 2 in 10 days; 1 each in 11, 15, 17, and 18 days. On the 36th day the 3 surviving mice were killed, of which 2 proved fecal carriers. The bacillus was not found in the spleen or gall bladder.

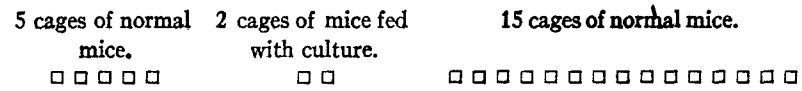
Series C.—The day after the feeding of the culture in milk just described, 13 mice (Series C) were placed in 2 cages of 5 and 8 respectively, and placed beside the cages containing the fed animals. They were all cared for by the same attendant. Of the cage of 5 mice, 1 only succumbed to mouse typhoid and that on the 12th day. Of the other cage, all 8 succumbed, on the 11th, 13th, 14th, 15th, 17th (2), 19th, and 25th days respectively. The unequal distribution of deaths in this series need not be considered now; but the main point is that already the culture Mouse Typhoid II had exhibited power to induce fatal infection through mediate contact as well as through direct feeding. Moreover, the contact mice succumbed in periods not exceeding those of certain of the fed mice.

Series D.—13 days after the feeding experiment of November 2, 24 mice in 8 cages (Series D) were placed beside the cages containing the 11 surviving mice of Series C. 11 died and 8 yielded positive cultures. At the expiration of 60 days, the remaining 13 were killed. 5 of them proved fecal carriers.

Experimental Epidemic.

The culture Mouse Typhoid II was now regarded as probably capable of producing mouse typhoid by ingestion in a large proportion of the fed mice and also of inducing that disease in exposed or contact mice not directly fed. Whether it possessed also the particular qualities which might be required in order that the spread from animal to animal should take place in the manner common in epidemics remained to be ascertained. An experiment to test this point was next designed.

Series E.—December 12, 1919. Food was withheld for 24 hours from 10 mice. Each mouse was then given milk to drink containing a heavy suspension of culture Mouse Typhoid II. It is estimated that each animal received approximately 1:20 of an 18 hour agar slant culture. At the end of 24 hours the mice were transferred to clean cages, 5 in each. With this experiment in view, 100 normal mice were assembled on December 8, into 20 cages, and arranged in positions indicated by the following diagram.



These mice had been free of fatalities. Just here it should be stated that the mice assembled for all the experiments were not only home-bred and of a stock free of disease, but when introduced into the experiment they were of an average weight of 13 gm. and age of 4 weeks.

The series of events were now as follows: Of the 5 mice in feeding cage 1, 2 died on the 8th, 1 each on the 13th, 14th, and 15th days. From all, cultures of Mouse Typhoid II were obtained. Of the 5 mice in feeding cage 2, 1 each died on the 12th, 13th, and 17th days. The 2 remaining mice were killed on the 60th day; cultures were negative.

Of the 100 mice in the 20 contact cages, the 1st animal died on the 15th day and the next on the 17th day. Within 60 days a total of 10 mice had succumbed. Of the 90 survivors, 7 (or 8 per cent) proved fecal carriers. Only 11 of the 20 cages showed infected mice as indicated either by death or carriage. The position of the mice, in respect to the purposely infected animals and the direction of the feeding or the cleaning of the cages, had no appreciable effect on the incidence of the infection. Obviously the attendant, in spite of more than ordinary precautions to keep his hands clean, early became contaminated and spread the contamination unwittingly and irregularly after the 1st day or two of his operations.

It is evident that what was produced in this experiment (Series E) was not an epidemic outbreak but rather a sporadic occurrence of mouse typhoid. But the impending conditions seem to have been significant. For on January 15, 1920, 79 normal mice in 17 cages (Series F) were assembled and placed on racks immediately next the cages of Series E on the 34th day after the beginning of that experiment. The effect was striking. No deaths occurred within the first 5 days, but there were 4 in the second 5 day period. The highest recorded were in the third 5 day period, and this wave quickly subsided. Two smaller waves appeared with their crests in the seventh and the ninth 5 day periods. The distribution of deaths in cages and periods is shown below.

Distribution of Deaths in Series F.

Days.	Deaths in cages.	Total No. of deaths in period.
1–4	0	0
5–9	Nos. 6, 13, 13, 16	4
10–14	" 1, 3, 4, 4, 5, 6, 7, 8, 9, 15	10
15–19	" 1, 2, 2, 4, 5, 6, 6, 10, 13	9
20–24	" 1, 4, 8, 11, 11	5
25–29	" 2, 3	2
30–34	" 2, 3, 11, 12, 15, 16, 17	7
35–39	" 2, 4, 13, 16	4
40–44	" 1, 8, 8, 8, 15	5
45–49	" 7, 13, 17	3
50–54	" 12, 14	2
55–65	" 13, 14	2

About 75 days after exposure the survivors of Series F, 24 in number, were killed and examined as carriers. 1 only was found. The following tabulation summarizes the results.

Total mortality	55, or	70 per cent.
Corrected "	41, "	52 " "
Cage attack rate	17, "	100 " "
Carriers among survivors	(1 in 24)	4 " "

A word of explanation is required regarding the terms total and corrected mortality. The latter refers to dead mice from which bacillus Mouse Typhoid II was recovered in cultures. The technique employed at this date was one in which the brilliant broth tubes were incubated for 1 hour before plating. Further studies showed that with this method many of these organisms are missed. This fact taken together with results obtained at a later date leads us to infer that the gross mortality figure as given is very nearly correct.

Thus it appears that once what may be termed sporadic cases of mouse typhoid are made to arise in a population previously free of this infection, the introduction of fresh, previously unexposed individuals may suffice, after a certain delayed or incubation period of 5 to 10 days, to bring about a sharp outbreak of cases which may reach epidemic proportions. In this particular instance every cage was attacked and the death rate very high.

Series G.—On February 13, 1920, or 29 days after the preceding or F series was started and at a time when the deaths among the latter had fallen, for the 5 days preceding, to 4, 48 normal gray tame mice of the average ages and sizes to the white mice employed up to this time, contained in 10 cages, were introduced into the mouse village and placed next the survivors of Series F. Instances of mouse typhoid arose among them and 25 mice died. On April 12, or 2 months after the placing of these mice in the village, the survivors were killed and examined for carriage of the bacillus Mouse Typhoid II. The following tabulation summarizes the results of this test.

Total mortality	25, or	52 per cent.
Corrected "	24, "	50 " "
Cage attack rate	10, "	100 " "
Carriers among survivors	(4 in 23)	17 " "

In brief, this series behaved very much as did the preceding one in respect to the several points covered in the study. It may be desirable

to point out the close correspondence between the total and corrected mortalities resulting from the improved bacteriological technique described on page 27.

It now became evident that it was possible to inaugurate epidemics of mouse typhoid among a healthy previously unexposed mouse population. Up to this time the events described are remarkably consistent. But as the experiment was continued by the successive introduction of fresh, vulnerable material, variations (or better stated perhaps wider fluctuations) made themselves apparent as we will now proceed to show.

At this point one or two incidental observations are called for. In the mouse room as arranged the racks carrying the cages were placed along the sides of two opposite walls, so that the cages could be made contiguous or could be separated by the distance of the width (12 feet) of the room. This separation did not in itself affect the closeness of contact, since in no instance did the mice actually mingle, but, as already stated, the intermediation of infected and non-infected animals was secured through the hands of the attendant.

However, the separation of cages by the width of the room made possible the carrying out of devices which might affect the mediation. For example, the attendant was made to use approved methods of hand sterilization[7] and to clean the cages and feed the mice on the side of the room away from the series in which the infection existed, before tending the latter.

Series H.—Thus on February 13, 1920, or the same day that Series G was brought into the village and placed in cages immediately adjoining those of Series F, 100 healthy mice were assembled in 20 cages (Series H) and placed by themselves on shelves along the opposite wall at a distance from those already in the room, where they were kept for 2 weeks. As deaths among these mice began within 2 weeks, it is evident that the carrying out of sterilization by the attendant did not suffice to render his hands free of contamination with the strain of bacillus employed in the infection experiments. The series was now brought over and placed next to Series I, described below. Deaths among them continued so that at the end of 2 months, April 12, the results were as follows:

[7] The cleaning of the hands of the attendant was carried out in the following order. 10 minutes scrubbing with hand brush, using tincture of green soap. Wash in warm tap water. 2 minutes in potassium permanganate solution. Then immerse in saturated solution of oxalic acid until stain is removed (3 to 5 minutes). Rinse in tap water.

Total mortality	33 per cent.
Corrected "	25 " "
Cage attack rate	(14 in 20) 70 " "
Carriers among survivors	(8 " 67) 12 " "

The lowered death rate and the reduced cage attack rate are at once apparent. That the events summarized in the tabulation accompanying Series H are not mere exceptions is shown by the next experiment.

Series I.—February 18, 1920. 177 healthy mice, distributed in 36 cages, were brought into the village and placed adjoining and in line with Series G. These healthy animals also were tended without sterilization of hands and tools, after Series G, in which the infection was still proceeding. The detached Series H was cared for first of all. It was supposed that should the infection appear among the mice of Series I, it would attain a degree of activity capable of being accelerated by the available mass of new infectible material introduced at about the period of the preliminary outburst, while the mice in the detached Series H might acquire some resistance due to slow infiltration of virus. As a matter of fact, the two series, H and I, behaved quite as if they had been one series and all the mice had been brought into the village at one time. The experiment involving Series I was terminated on May 18, or after 3 months. The tabulation gives the final outcome.

Total mortality	64, or 36 per cent.
Corrected "	47, " 26 " "
Cage attack rate	(28 in 36) 80 " "
Carriers among survivors	(14 " 113) 12 " "

Review.

It is not our intention to enter into a minute discussion in this place of the significance of these experimental data. Such discussion as we purpose to give the subject will be presented in connection with the next paper in which the succession of events taking place day by day in the several series will be described.[8] We prefer merely to pass in review in this place the salient facts connected with the experiments detailed.

The feeding of the mice exposed in the village and the cleaning of the cages were done by one person and always in the same direction.

[8] Amoss, H. L., *J. Exp. Med.*, 1922, xxxvi, 45.

As each new series was introduced, its cages were first tended. Series G and I were brought into the mouse village when the epidemic in Series F was at the crest of the wave. Series F (79 mice) was assembled and exposed on January 15, Series G (48 mice) on February 13, Series H (100 mice at distance until February 27) on February 13, Series I (177 mice) on February 18. Series F, G, and I were contiguous; Series H was separated by the distance (width) of the room (12 feet). Before the cages of Series H were touched, the attendant scrubbed his hands and nails with soap and brush for 10 minutes and used permanganate of potash and oxalic acid as disinfecting solutions. After 2 weeks separation Series H was brought into contact with Series I.

The several series may be regarded as having been placed in a single line, with the cleaning and feeding carried out in one direction. Thus following Series F there are 10 cages of Series G containing 5 mice each, followed by 36 cages of Series I and 20 cages of Series H, also of 5 mice each. Numbering the cages in order, Series G would be covered by cages 1 to 10; Series I by cages 11 to 46; and Series H by cages 47 to 66 inclusive. It might be expected that mice would be first attacked and die in cages 1 to 5 and that the infection would spread by contiguity. But the order is not so regular as this and the contrary may happen, for the first mice to die of mouse typhoid were in cage 7; then in cages 2, 3, and 4; then in Nos. 2, 3, and 5; then in Nos. 9 and 10. In Series I, of 36 cages, exposed 5 days after Series G, the distribution was as follows:

Days.	Deaths in cages.
1–4	Nos. 20, 29
5–9	" 11, 16, 17, 23, 25, 34
10–14	" 18, 24, 27, 28, 33, 34, 39
15–19	" 11, 13, 14
20–24	" 13, 23, 30, 32, 34
25–29	" 13, 26, 32, 33
30–34	No. 30
35–39	Nos. 26, 27
40–44	" 24, 29
45–59	" 11, 19, 21, 24

The one safe deduction from this tabulation seems to be that a wide but not uniform distribution of the bacillus is quickly brought about by the attendant through which individual mice, in an entirely unpredictable order, take it up and fall victims to the infection ensuing. Once this has happened the cages must soon become contaminated widely by the excrement carrying the bacillus and all the remaining mice should receive the organism. The sources of the subsequent irregular events can only be inferred as depending upon such factors as dosage and possibly fluctuations in pathogenic activity of the bacillus, and upon variations in the resisting powers of the mice. These factors are the ones commonly invoked to explain such vagaries of case incidence of the communicable diseases as here presented and are set down here not as finalities or even as matters for discussion, but rather to emphasize a parallelism existing between the natural, so called, infections in man and animals and those purposely set up, as in the instance being considered, which may be used in putting the next question to be answered by experiment.

In concluding this presentation, a table has been prepared of the five series of mice exposed to infection with the bacillus of mouse typhoid (Table I). The number of mice in each series varied from 48 to 177. In the table, Series I, which contained the largest number given, is broken up into three parts called first, middle, and last, according to the degree of removal of the cages at the time they were brought into the mouse village from the mice which were already potentially infected. It is not suggested that the factor of nearness or remoteness is a controlling one, but the division is interesting as bringing out again the element in the process of distribution of the infection which for the present is merely termed vagary. It is to be kept in mind that the quality of mice in the three divisions was superficially homogeneous. The only mice fed with culture were 10 of Series E. The rest of the animals received the bacillus through the exigencies of contact between them, their habitation, and the hands of the attendant.

The figures in Table I not only speak for themselves but have already been discussed in the earlier pages of this paper. The exception is Series I in which the three groups of approximately 60 mice each, selected by degree of removal from the older infected series into

relation with which they were brought, show a mortality of 38, 56, and 15 per cent respectively. Since the first group of 60, immediately next the older and already infected series, gave a lower mortality than the middle and further removed group, the very low mortality in the last group can hardly be accounted for by position alone.

TABLE I.

Composite Table of the Five Series.

Series.	No. of mice in series.	Mortality.	Corrected mortality.	Carrier rate among survivors.	Carrier rate in total No.	Cage attack rate.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
E*	100	10	10	8	7	55
F	85	70	52	4	1	100
G	48	52	50	17	8	100
I First	60	38	22	11	7	83
I Middle	60	56	52	4	2	100
I Last	57	15	5	18	15	50
H	100	33	25	15	10	70

* The infection in this series was produced by feeding with a suspension of *B. typhi murium* 10 of the mice.

The tabulation brings out a striking irregularity between carrier and death rate. Thus of 14 carriers, 8 were found in the same number (8) of cages in which no deaths from mouse typhoid took place, while 6 were found in 29 cages in which deaths from the infection took place. The distribution of carriers according to deaths in the cages is as follows:

No. of deaths in cage.	Instances of carriage of bacillus.
0	8
1	3
2	1
3	2
4	0

Experiments with Strains Arising from a Single Bacillus.

Six strains were obtained from single cells of Mouse Typhoid II by Barber's method. In order to be certain that only single cells

were picked, the strains obtained were plated, a colony was picked, and from the 8 hour growth in broth a single cell was again isolated and the same process repeated. Thus before a strain was considered as arising from a single cell, the culture was analyzed by the Barber method three times.

Six such pure-line strains were obtained, three of which showed slightly greater virulence by intraperitoneal injection into mice, as shown in Table II.

TABLE II.

Results of Intraperitoneal Injection of 1:30,000 of a Culture of Each of Six Single Cell Strains of Mouse Typhoid II.*

Weight of mice.	Length of life.					
	Strain.					
	A	D	E	F	G	H
gm.	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*
13	52	44	66	20	66	64
	60	60	78	54	72	66
14	75	78	100	64	84	72
	88	78	105	66	90	78
15	80	82	115	72	94	100
	90	86	134	78	134	138
16	96	94	150	84	150	200
	111	110	160	99	200	255
17	130	130	255	115	408	260
	140	132	S.†	200	520	495

* The suspensions were standardized in a turbidimeter.

† S. indicates survived.

Comparison of the Virulence of Single Strain A with the Composite or Original Strain of Mouse Typhoid II.—Since the results of intraperitoneal injections (virulence tests) of mice with the mouse typhoid organisms vary considerably, it becomes necessary to use large numbers of mice in each experiment. With the view of determining what mice grouped according to weight would yield the most concordant results, the following experiment was made.

Experiment 1.—Mice were selected from a large stock until 10 of each weight from 10 to 17 gm. were obtained. Three lots of 6 each, composed of individuals weighing 18, 19, and 20, and 1 weighing 21 gm., were selected. Each of the 99 mice received intraperitoneally in 1 cc. of salt solution 1:30,000 of a 16 hour slant agar culture of Mouse Typhoid II. This strain had been plated repeatedly so that the culture represents the descendants of a single colony.

The number of hours before death is recorded for each mouse in Table III.

TABLE III.

Relation of Weight to Susceptibility to Intraperitoneal Injection of Mouse Typhoid, Strain II.

November 30, 1920. Each mouse received intraperitoneally in 1 cc. 1:30,000 of a 16 hour growth on pH 7.4 agar; suspension standardized in a turbidimeter.

Length of life.											
Weight of mice.											
10–10.5 gm.	11–11.5 gm.	12–12.5 gm.	13–13.5 gm.	14–14.5 gm.	15–15.5 gm.	16–16.5 gm.	17–17.5 gm.	18–18.5 gm.	19–19.5 gm.	20–20.5 gm.	21–21.5 gm.
No. of mice.											
10	10	10	10	10	10	10	10	6	6	6	1
hrs.	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*
20	20	20	20	49	20	20	20	46	20	20	89
20	20	27	25	66	30	59	20	73	60	20	
25	20	28	40	72	52	64	20	86	70	30	
48	41	60	50	75	52	86	27	106	75	60	
50	63	60	65	78	60	89	30	115	91	67	
69	67	72	74	79	73	90	34	130	96	75	
86	67	83	75	86	90	118	75				
98	67	86	89	115	115	118	86				
106	86	93	93	125	130	118	88				
110	113	104	106	160	144	160	90				

The number of deaths recorded in each group at 50, 75, 100, and 150 hours shows little correspondence, so that no conclusion can be reached in the matter of the selection of the most suitable group for testing virulence. If, however, a single cell strain of this micro-organism is employed, there appears in the same kind of experiment a definite relation between the susceptibility and age groups, as is shown in Experiment 2.

Experiment 2.—120 mice were selected so that there were groups of 10 of each weight from 10 to 21 gm. Such mice received intraperitoneally in 1 cc. 1: 30,000 of a 16 hour slant agar growth of single cell strain Mouse Typhoid II A. The suspensions were standardized in a turbidimeter to the density of the suspension used in Experiment 1. The results are shown in Table IV.

TABLE IV.

Relation of Weight to Susceptibility to Intraperitoneal Injection of Single Cell Strain Mouse Typhoid II A.

January 13, 1921. Each mouse received intraperitoneally in 1 cc. 1:30,000 of a 16 hour growth on pH 7.4 agar; suspension standardized in a turbidimeter.

Length of life.											
Weight of mice.											
10–10.5 gm.	11–11.5 gm.	12–12.5 gm.	13–13.5 gm.	14–14.5 gm.	15–15.5 gm.	16–16.5 gm.	17–17.5 gm.	18–18.5 gm.	19–19.5 gm.	20–20.5 gm.	21–21.5 gm.
No. of mice.											
10	10	10	10	10	10	10	10	10	10	10	10
hrs.	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*
50	20	26	14	20	20	40	70	16	18	96	90
58	20	28	16	60	58	58	86	75	90	124	117
64	22	58	52	67	60	64	88	90	96	136	168
66	26	68	70	70	74	70	90	106	132	150	168
70	34	70	74	85	75	80	111	117	134	188	186
74	42	74	106	100	88	90	132	142	158	202	206
85	52	90	114	132	148	124	130	184	266	248	208
90	100	96	122	132	168	140	140	230	304	260	260
154	108	108	140	150	254	312	250	264	308	296	404
158	115	115	180	163	256	S.	266	S.	624	404	630

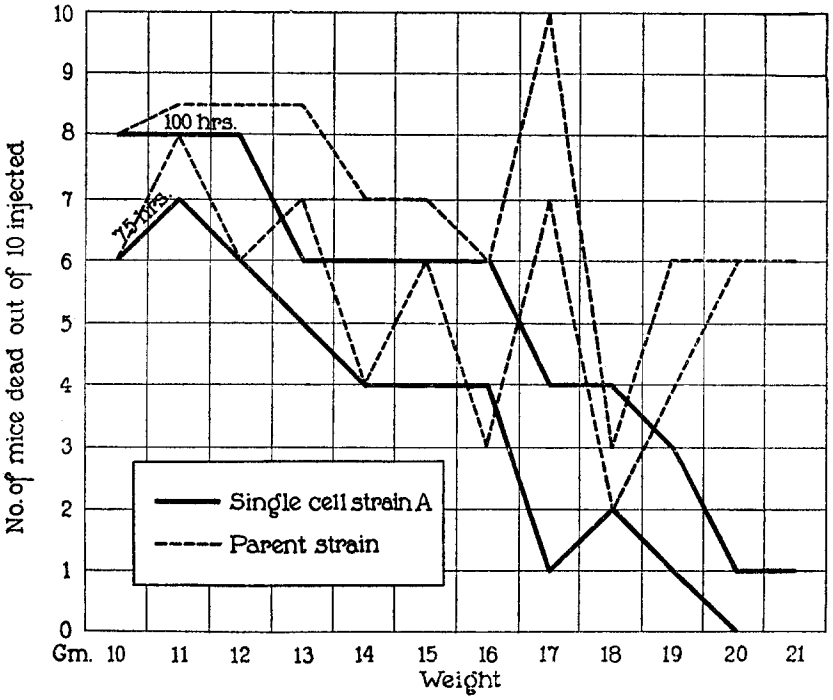
A comparison of the isolethal lines at 75 and 100 hours in Experiments 1 and 2 is shown graphically in Text-Fig. 1.

Of the two, the parent strain seems to be more virulent. The irregularity of the results in Experiment 1 and the obvious correlation between body weight and susceptibility shown in Experiment 2 with the single cell strain point to individual variation among the microorganisms comprising the parent strain.

Pathogenicity of Single Cell Strains Administered by Mouth. Experiment 3.—The power to infect by mouth of three of the single cell strains was tested by allowing groups of 8 mice each to drink milk containing living cultures of the strain to be

tested. Of 8 mice receiving Strain A, 5 died and 3 were living after 30 days; with Strain F 6 mice died and with Strain H only 2 died. Strain F was therefore selected in the experiment to be described, in which attempts were made to start an epidemic of mouse typhoid under the conditions known to be favorable.

Series O. Experiment 4.—February 10, 1921. Each of 10 normal mice from which food had been withheld for 24 hours was fed by stomach tube 1:250 of a 16 hour slant agar growth of pure-line Strain F.



TEXT-FIG. 1. Comparison of the relation of the virulence of a single cell strain and of the parent strain to body weight of mice. The injections were made intraperitoneally. The difference in weight of the 10 mice in a given group was not more than 0.5 gm.

February 11. The 10 mice in 2 cages, 5 in each, were placed midway on a shelf among 20 other cages, each containing 5 normal mice. During the following 46 days, 6 of the feeders and 7 of the contacts died of mouse typhoid.

On the 46th day of observation 100 normal mice in 20 cages (Series P) were brought into line with Series O. There was no sharp increase in deaths among the normal mice added or among the original. Thus among the new mice (Series P) the first death from mouse typhoid occurred 3 days later, and one on the 14th,

18th, and 36th days. Within 98 days only 11 of the mice died. During this period 10 of Series O died of mouse typhoid, making a total of 17 of the contacts in Series O and 6 of the feeders.

Since the addition of fresh normal mice in the above experiment with pure-line Strain F did not result in an outbreak of mouse typhoid under conditions which in our experience constantly incite epidemics with Mouse Typhoid II, another pure-line strain was employed in the next experiment under slightly different conditions; *viz.*, the new mice were added on the 31st day as in Series K, L, and M[9] instead of the 46th day as in Series O and P.

Series Q. Experiment 5.—June 8, 1921. Each of 10 normal mice from which food had been withheld for 24 hours was allowed to drink milk containing 1:20 of a 16 hour agar slant growth of pure-line Strain A, isolated from Mouse Typhoid II and belonging to the three more virulent pure-line strains.

On the following day the mice were placed in 2 cages, 5 in each, situated midway in a line of 20 cages, each containing 5 normal mice. During the following 30 days only 2 of the contact mice died. None of the feeders succumbed. On the 31st day, 100 normal mice in 20 cages (Series R) were added in line with Series Q. During the next 112 days only 7 of these mice died.

Comment.—Pure-line Strains F and A came from single cells picked from Mouse Typhoid II, the strain which was used to start the long replacement series, K, L, and M. It will be seen in another paper in this issue[8] that in the latter series the addition of new normal mice during a quiescent period was followed shortly by a new outbreak, first among the new or added mice and then among the old mice; that is, mice which had been for some time exposed to the virus. Under these same conditions of feeding, arrangement, and time under which the epidemic waves in Series K, L, and M were established with Mouse Typhoid II, the pure-line strains signally failed to induce an epidemic. Thus the individuals from which Strains F and A descended lacked the power to produce an epidemic under the same conditions which sufficed for the composite strain Mouse Typhoid II to incite an epidemic outbreak.

[9] These series are described at length on p. 46 of this issue.

SUMMARY.

In this paper we have described the first part of an experimental study of the epidemiology of mouse typhoid. One set of data has been presented on the basis of which little or no analysis has been attempted. The immediate object has been rather to collect materials than to undertake to account for the phenomena encountered. It is obvious that the factors involved in the inquiry are intricate, but it is believed that they are not necessarily or all beyond disentanglement. About 500 mice in all have been studied in an experimental village, brought together in increments among a population in which mouse typhoid experimentally induced was prevailing.

The results have been presented according to two phenomena; namely, mortality or death rate, and bacillus carriage rate. The material does not lend itself to consideration according to morbidity rates. If it were established that every instance of attack, when not fatal, was attended by carrier production for the bacillus of mouse typhoid, reliable morbidity tables could be constructed. In the absence of this certain criterion, the materials here presented can be dealt with only as mortality data. This fact is attended with obvious disadvantages in respect to the epidemiological material assembled regarding infectious disease in man. In spite, however, of the drawbacks, it is already evident that the results obtained by the sort of inquiry here described may come to throw no inconsiderable light on moot problems on the origin, mode of spread, and manner of decline of epidemic diseases in general.

The analysis of the strains by selecting single cells and thus establishing substrains has yielded results which may eventually have value in explaining fluctuations in virulence. Among the positive data arising from the experiments with such cultures are, first, that there have been obtained by mechanical means from the ordinary bacteriologically pure culture, single cell strains exhibiting slightly different pathogenic activity, whether administered by mouth or parenterally, and second, that more regular results are obtained with intraperitoneal injections of these strains than with the parent strain. Among the negative results to be recorded are the failures of two single cell strains to incite an epidemic among mice under conditions known to be suitable when the parent strain is used.